Pestic. Sci. 1997, **50**, 91–98

Seasonal Dynamics of Metabolic Mechanisms Mediating Pyrethroid Resistance in *Helicoverpa armigera* in Central India

Keshav R. Kranthi,[1] Nigel J. Armes,[2] Nagarjun G. V. Rao,[1] Sheo Raj[1] & Veerakeralam T. Sundaramurthy[1]

[1] Central Institute for Cotton Research, Post Bag No. 225, GPO, Nagpur 440 001, India
[2] Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK

(Received 18 December 1995; revised version received 16 July 1996; accepted 18 February 1997)

Abstract: Very high cypermethrin and fenvalerate resistance frequencies were recorded in *Helicoverpa armigera* (Hübner) populations in central India during the 1993–94, 1994–95 and 1995–96 cropping seasons. Synergism assays and biochemical analyses of detoxification enzyme levels indicated that monooxygenases and esterases were important metabolic mechanisms mediating pyrethroid resistance. Piperonyl butoxide- (PBO) and profenofos-suppressible pyrethroid resistance were correlated with enhanced levels of cytochrome P450 and general esterases respectively. Enzyme assay data indicated that high cytochrome P450 levels generally coincided with low esterase activity and *vice versa*. Similarly, synergist bioassays showed that PBO-insensitive resistance was frequently associated with profenofos-sensitive resistance and *vice versa*. Oxidase- and esterase-mediated mechanisms evidently alternated in a reciprocal manner, with perceptible shifts in relative importance occurring during mid-October in all three seasons and in late January in 1995. Apart from metabolic mechanisms, a synergist-insensitive resistance mechanism (believed to be nerve insensitivity), accounted for an average of 51, 30 and 28% of cypermethrin resistance during the 1993–94, 1994–95 and 1995–96 seasons respectively.

Key words: Insecta, *Helicoverpa armigera*, pyrethroids, insecticide resistance, resistance mechanisms

1 INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a major pest of cotton, legumes and tomatoes in the Old World tropics. In India, crop losses due to *H. armigera* are considerable, commonly more than half the yield, and annual losses to cotton and pulses alone have been estimated at US$300–500 million.[1] In recent years, management of *H. armigera* has become increasingly difficult due to the development of resistance to all the major insecticide classes available to Indian farmers for bollworm control.[2,3] Pyrethroid resistance levels are particularly high, not only in India,[2–4] but also in Australia,[5] Indonesia[6] and Thailand,[7] significantly decreasing the efficacy of this group of insecticides against *H. armigera* in most of Asia and Australia. In India, an intensive country-wide resistance monitoring programme has demonstrated that pyrethroid resistance is now widespread across the country and significant levels of cyclodiene and organophosphate resistance are also present in most populations.[8]

Several mechanisms of resistance have been identified in *H. armigera* populations in various parts of the world, including reduced penetration,[9,10] decreased nerve sensitivity[9,11] and enhanced metabolism.[9,12] In Indian populations, mechanisms have not been studied exhaustively. Nerve insensitivity has been shown to be present in varying degrees in field populations[11] and metabolic resistance has been inferred from synergist studies.[8,13]

The purpose of this study was to determine the current status and dynamics of cypermethrin and fenvalerate resistance in *H. armigera* in central India and to

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

investigate the metabolic mechanisms underlying this resistance through biochemical assays.

2 MATERIALS AND METHODS

2.1 Insects

From one to three thousand *H. armigera* eggs were collected weekly from a range of crops in farmers' fields and the farm of the Central Institute for Cotton Research, within a 60 km radius of Nagpur, Maharashtra, between September and April, during three cropping seasons between 1993 and 1996. The major sources of eggs were: cotton from September to December and pigeonpea, soybean, chickpea and the wild hosts *Lagascea mollis* Cav. (Asteraceae) and *Chenopodium album* L. (Chenopodiaceae), from January to the end of April. Eggs were transferred individually to 7·5-ml cells of 12-well tissue culture plates containing a chickpea-based semi-synthetic diet.[14] All rearing and bioassay operations were carried out at 25(±2)°C under a 12 : 12 h light : dark regime.

2.2 Insecticides and chemicals

The following technical grade insecticides were used for bioassays: *cis : trans* (*c.* 50 : 50 ratio) cypermethrin (900 g kg^{-1}; Zeneca Agrochemicals, UK); fenvalerate (976 g kg^{-1}; Sumitomo Corp., Japan); profenofos (940 g kg^{-1}; Ciba Geigy, Switzerland). Piperonyl butoxide (PBO) (900 g kg^{-1}), was obtained from Goodeed Chemical Co., UK. All other chemicals were of high purity and obtained either from Sigma Chemicals, USA or Hi-media Chemicals, Bombay.

2.3 Bioassays

Once the larvae attained 30–40 mg (third–fourth-instar), they were randomly assigned to the following topical application treatments: cypermethrin 0·1 μg per larva, fenvalerate 0·2 μg per larva (approximations to the LD_{99} values for pyrethroid-susceptible *H. armigera*);[5,8,15] cypermethrin 1·0 μg per larva (used as a 'twin' cypermethrin dose because of high survival to cypermethrin 0·1 μg per larva).[8,15] In addition, the following synergists were applied to larvae in combination with cypermethrin 0·1 μg: PBO 50·0 μg per larva (to determine the extent of PBO-suppressible pyrethroid resistance);[5,8,15] profenofos 0·1 μg per larva (to determine the extent of profenofos-suppressible pyrethroid resistance);[5] *S,S,S,*-tributylphosphorotrithioate (DEF) 20·0 μg per larva (to estimate the role of esterases in pyrethroid resistance);[12] diethyl maleate (DEM) 50·0 μg per larva (to estimate the role of glutathione-*S*-transferases (GST)[5] and triphenyl phosphate (TPP) 50·0 μg per larva (to estimate the role of carboxylesterases).[5] PBO and profenofos were applied as pre-mixes with the cypermethrin. DEF, DEM and TPP, which were only evaluated between November 1994 and April 1995, were applied 15–20 min prior to the cypermethrin. When applied alone, none of the synergists caused mortality (Kranthi, unpubl. data).

Each week, at least 100 (usually 120–250), larvae were treated topically on the dorsal thorax with 1·0 μl of one of the insecticide treatments. Larval mortality was assessed at six days after treatment as described by Armes *et al.*[3,8]

2.4 Enzyme preparations

At monthly intervals, three replicates each of at least twenty 80–100-mg, fourth-instar, larvae resulting from the regular egg collections, were dissected and their midguts removed. Dissections were carried out in ice-cold sodium phosphate buffer (100 mM; pH 7·6) containing potassium chloride (11·5 g $litre^{-1}$). Fat body and food particles were removed from the midguts which were then homogenised individually in fresh sodium phosphate buffer containing 1 mM each of EDTA, phenyl thiourea and phenyl methyl sulfonyl fluoride and glycerol (200 g $litre^{-1}$). The homogenate was centrifuged at 10 000*g* for 15 min at 0°C and the resultant post-mitochondrial supernatant used as the enzyme source. Protein was estimated according to Lowry *et al.*,[16] using BSA (Type V), as standard.

2.5 Cytochrome P450 determination

Cytochrome P450 was determined from the dithionite-reduced CO difference spectrum method described by Omura and Sato,[17] using a molar extinction coefficient of 91 mM^{-1} cm^{-1}.

2.6 Esterase determination

Five millilitres of 0·3 mM α-naphthyl acetate in 40 mM sodium phosphate buffer (pH 7·0) was incubated with 1·0 ml of gut homogenate supernatant containing 5 μg of protein, at 30°C for 10 min. The reaction was stopped by addition of 1·0 ml of a freshly prepared aqueous solution containing fast blue BB salt (2·8 g $litre^{-1}$) and sodium dodecyl sulphate (35·7 g $litre^{-1}$). Absorbance at 590 nm was read against blanks and the activity quantified using α-naphthol as standard.

2.7 Glutathione-*S*-transferase determination

The assay mixture consisted of 50 μl of 50 mM 1-chloro-2,4-dinitrobenzene (CDNB) (in 95% ethanol), 150 μl of 50 mM reduced glutathione (GSH) and an aliquot (3·0 ml) of enzyme source containing protein (80 μg) in sodium phosphate buffer (100 mM; pH 7·6) and phenyl

thiourea (0·1 mM). The assay was conducted at 25(±1)°C. Change in absorbance at 340 nm was recorded and the amount of GSH conjugate formed was calculated using an extinction coefficient equal to 9·6 mM^{-1} min^{-1}.

A double beam UV temperature-controlled spectrophotometer (Hitachi U-2000), was used for protein estimation and all enzyme assays. Enzyme activity is expressed as per mg protein.

3 RESULTS

3.1 Pyrethroid resistance

Both cypermethrin and fenvalerate resistance frequencies were high throughout the three cropping seasons (Fig. 1). Resistance to cypermethrin 0·1 μg ranged from 47 to 97% (average 79%) during 1993–94, 71–100% (average 88%) during 1994–95 and 65–99% (average 88%) during 1995–96. In 1993–94, a decrease in resistance frequencies was observed between August and November, but frequencies then steadily increased over the remainder of the season. In 1994–95 and 1995–96, overall seasonal changes were less obvious, except for a tendency for slightly lower resistance in October–November and toward the end of each season. The fenvalerate 0·2 μg resistance profiles were similar to those of cypermethrin 0·1 μg during the three seasons (average 72%, range 40–95% in 1993–94; average 87%, range 58–100% in 1994–95 and average 84%, range 63–99% in 1995–96), although the decrease in resistance frequencies during August–November 1994 was more pronounced in the fenvalerate treatment. Cypermethrin 1·0 μg resistance frequencies were significantly higher in 1994–95 and 1995–96 than in 1993–94 (average 51%, range 31–79%; average 56%, range 32–75% and average 23%, range 6–48% respectively).

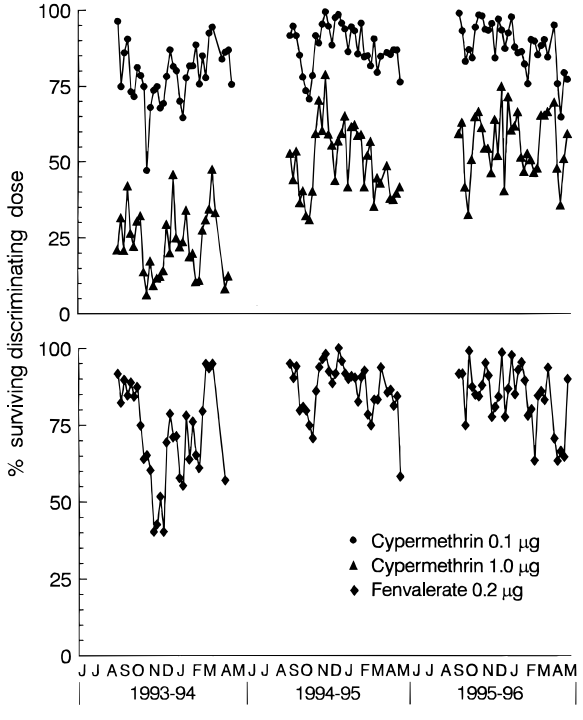

Fig. 1. Weekly pyrethroid resistance frequencies in *Helicoverpa armigera* from the Nagpur region during the 1993–94 and 1994–95 cropping seasons. Results given as the percentage of 30–40-mg larvae surviving the specified discriminating dose of insecticide.

3.2 Pyrethroid synergism

Weekly data on the impact of PBO and profenofos on the suppression of cypermethrin resistance are presented in Fig. 2. Up to the end of October 1993, Pbo suppression averaged 38%, after which it decreased substantially to an average of only 4% for the remainder of the 1993–94 season. Similarly, there was a marked change in profenofos-suppressible resistance over the same period. Prior to the first week of October 1993, profenofos suppression averaged only 6%, but then increased significantly to an average of 48% for the remainder of the season. The final collections, made in late March–mid April, recorded reduced profenofos suppression, averaging 18% without a concomitant increase in PBO suppression. This suggests a change in the relative importance of resistance mechanisms

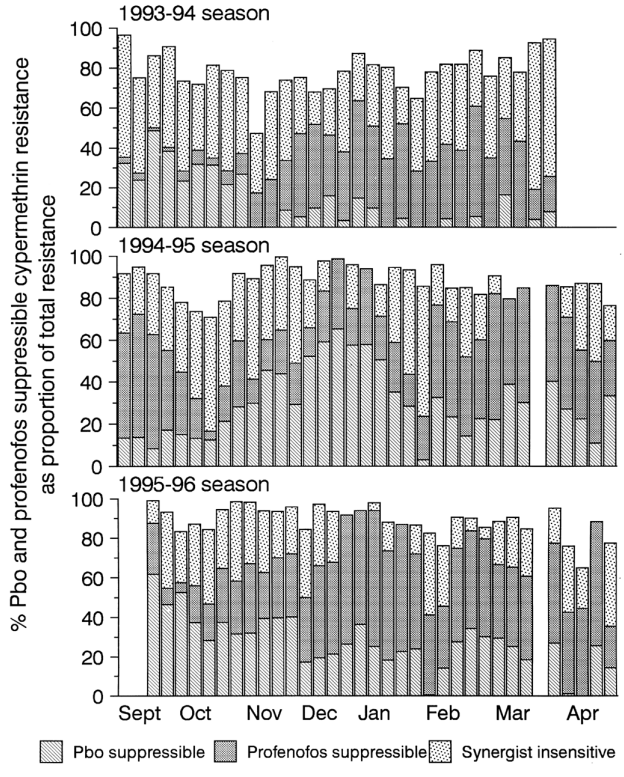

Fig. 2. Relative contributions of PBO-suppressible, profenofos-suppressible and synergist-insensitive mechanisms to weekly cypermethrin resistance frequencies in *Helicoverpa armigera* from the Nagpur region during the 1993–94, 1994–95 and 1995–96 cropping seasons. Results given as the percentage of 30–40-mg larvae surviving cypermethrin 0·1 μg per larva alone or in combination with PBO at 50 μg per larva or profenofos at 20 μg per larva.

toward a third, possibly non-metabolic (synergist-insensitive) mechanism.

During the 1994–95 season there was a switch-over of metabolic mechanisms in mid-October from a more prominent profenofos-suppressible mechanism (average 47% up to mid-October *cf.* 20% from mid-October to late January), to a PBO-suppressible mechanism (average 16% up to mid October *cf.* 44% from mid-October to late January). A second switch in relative importance of mechanisms from PBO-suppressible to profenofos-suppressible resistance, occurred in late January with profenofos and PBO suppression averaging 51 and 29% respectively. Unlike the 1993–94 results, these changes in synergist suppression were not indicative of a total quantitative shift toward one particular metabolic mechanism, but rather of equal importance, with both mechanisms simultaneously mediating resistance to some extent. As in 1993–94, there was a significant non-synergisable resistance component in most weeks.

During the 1995–96 season, up to the end of November 1995, PBO suppression of cypermethrin resistance averaged 35% compared with profenofos suppression of 21%. From December onwards, PBO suppression fell to an average of only 19%, whilst profenofos suppression increased to 42%. This shift in synergist sensitivity patterns was more or less analogous to the 1993–94 observations. Similarly, at the end of the cropping season in April there was some evidence of a shift in mechanisms towards a synergist-insensitive resistance.

During the 1994–95 season, the metabolic inhibitors DEF, DEM and TPP did not indicate any significant synergism of pyrethroid toxicity (Fig. 3). In fact, from February to April 1995 all three inhibitors (with the exception of DEM in April), were consistently moderately antagonistic.

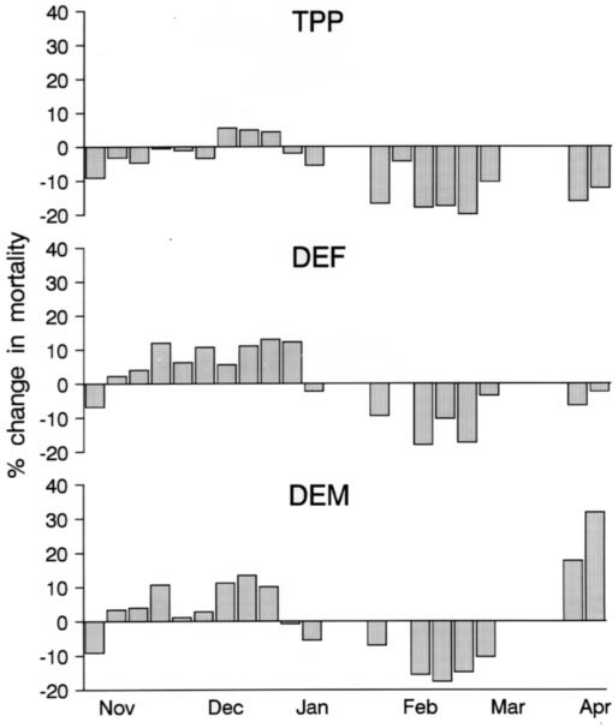

Fig. 3. Impact of three synergists in combination with the cypermethrin 0·1 μg per larva discriminating dose on mortality of 30–40-mg *Helicoverpa armigera* larvae from the Nagpur region between November 1994 and April 1995 (positive change in mortality indicates synergism, negative antagonism).

3.3 Detoxification enzyme activity

The seasonal changes in cytochrome P450, GST and esterase titres are presented in Fig. 4.

Between September and October of the 1993–94 season, cytochrome P450 and GST titres were relatively high (462–478 pmol mg^{-1} protein and 141–157 μmol min^{-1} mg^{-1} protein respectively), and esterase activity low (11–13 μmol mg^{-1} protein), compared to the enzyme titres observed in the later part of the season. Larvae collected as eggs from the field between November 1993 and March 1994 showed reduced cytochrome P450 and GST titres, ranging from 192 to 312 pmol mg^{-1} and 107 to 124 μmol min^{-1} mg^{-1} respectively, and concomitant high esterase activity of 36–54 μmol mg^{-1}.

A very similar pattern of enzyme changes was evident during the 1995–96 season, with high cytochrome P450 and GST titres (348–406 pmol mg^{-1} and 133–140 μmol min^{-1} mg^{-1} respectively), and low esterase activity (ranging from 7 to 12 μmol mg^{-1}), during September–October, followed by lower cytochrome P450 and GST titres (163–254 pmol mg^{-1} and 93–112 μmol min^{-1} mg^{-1} respectively) and increased esterase activity (17–45 μmol mg^{-1}) during the latter part of the season from November to April.

Though seasonal changes in enzyme activity were apparent during the 1994–95 season, the pattern was different from those in 1993–94 and 1995–96. Cytochrome P450 titres were low over most of the season, with the exception of the December–January period (peak titre, 353 pmol mg^{-1}). Esterase activity remained relatively low over most of the season (ranging from 14 to 29·9 μmol mg^{-1}). The pattern of changes in GST titres was similar to that reported for cytochrome P450, with high titres of 144–155 μmol min^{-1} mg^{-1} during December 1994–January 1995 and also in March 1995.

Comparing Figs 2 and 4, it is apparent that the observed seasonal patterns of cypermethrin synergism with PBO and profenofos were associated with similar changes in cytochrome P450 and esterase titres respectively. Low levels of PBO suppression of cypermethrin resistance (4–29%) during November 1993–March 1994, September–October 1994, February–March 1995 and December 1995–April 1996, were associated with low to moderate cytochrome P450 titres (range: 112–312 pmol mg^{-1}). Similarly, higher PBO suppression (38–44%) during September–October 1993, November 1994–January 1995 and September–November 1995

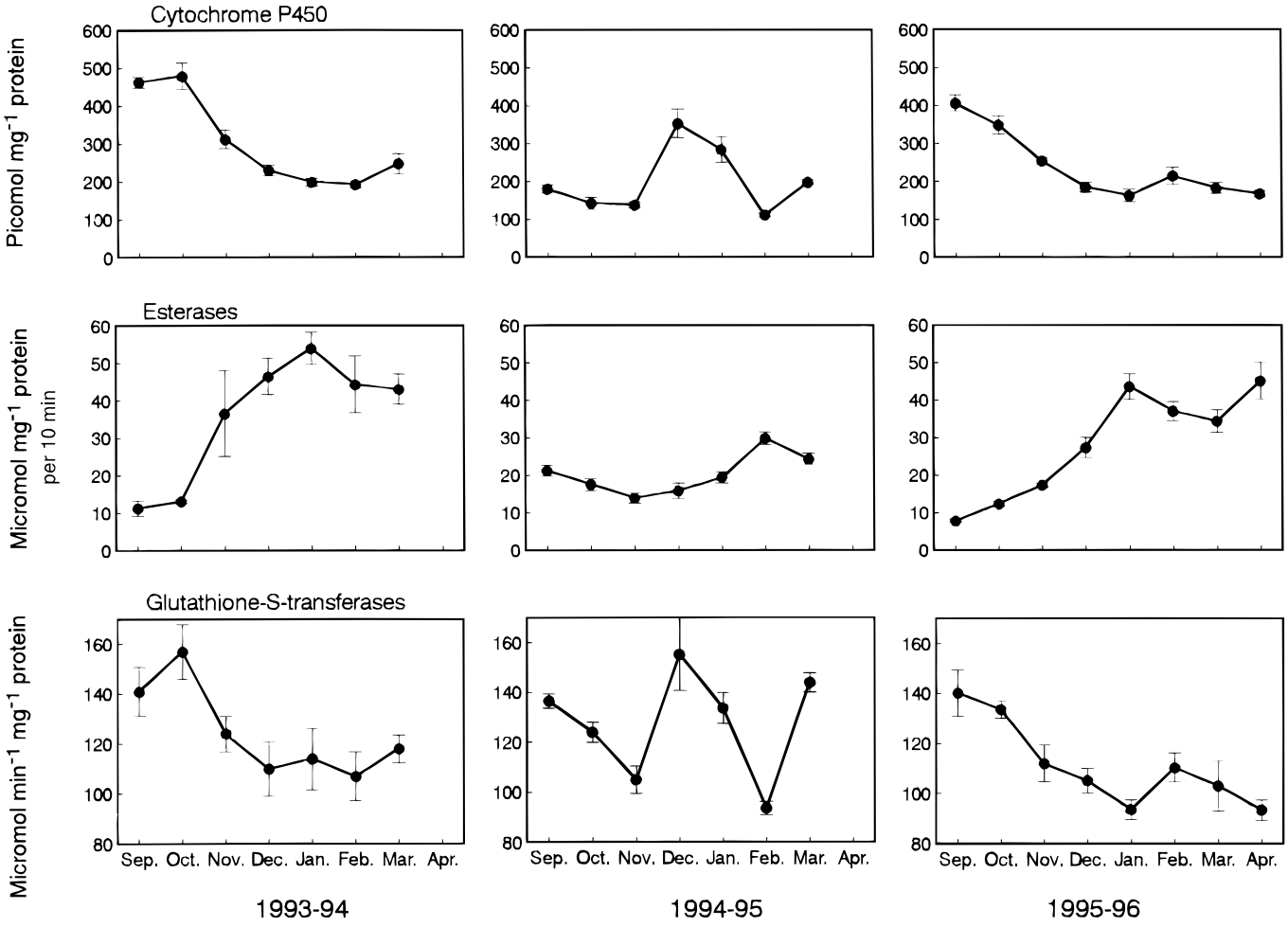

Fig. 4. Detoxification enzyme levels in fourth-instar *Helicoverpa armigera* larvae from the Nagpur region between September and April during the 1993–94, 1994–95 and 1995–96 cropping seasons. Error bars represent the S.E. of three experiments.

coincided with enhanced P450 titres (range: 284–478 pmol mg^{-1}).

In the case of profenofos-suppressible resistance, low suppression (6–21%) during September–October 1993, October 1994–January 1995 and September–November 1995 were associated with reduced esterase activity (range: 12–16 μmol mg^{-1}), and high suppression (42–51%) during November 1993–March 1994, September–October 1994, February–April 1995 and December 1995–April 1996, with enhanced esterase titres (range: 19–45 μmol mg^{-1}).

4 DISCUSSION

The very high pyrethroid resistance frequencies recorded in *H. armigera* in the Nagpur region were not expected, as pyrethroids had not been over-used on cotton in recent years, usually contributing, at most, two applications out of a total of six to eight sprays. Since the mid-1990s, endosulfan, monocrotophos, quinalphos and chlorpyrifos have become the preferred insecticides for bollworm control in the region. As the highest insecticide use against *H. armigera* is on cotton between August and November, it was anticipated that the most rapid increase in resistance frequencies would occur, if at all, between September and December, as moths resulting from insecticide-selected larvae mated and oviposited. To some extent the data support this, particularly in the 1994–95 season when the most rapid increase in pyrethroid resistance frequencies occurred in October–November. In the 1993–94 season, the data indicated that resistance increased slightly later, from November, and continued to increase until late March. This situation may have arisen because high *H. armigera* population pressure on crops such as pigeonpea and chickpea, which flower late in the season after most cotton crops in the region have senesced, necessitated more frequent insecticide application than usual to these crops up to late February. The decrease in resistance during September–October each year and at the end of each season was most likely due to little insecticide selection pressure on *H. armigera* populations taking place during, and two or three weeks prior to, these periods.[15]

The combined evidence from synergism bioassays and in-vitro enzyme assays clearly implies a significant

role of metabolic mechanisms in pyrethroid-resistant *H. armigera* from central India. Changes in the levels of synergism exhibited by PBO and profenofos were associated with similar patterns of changes in the activities of the corresponding enzymes. Although the extent of synergism exhibited by the inhibitors was not precisely associated with specific enzyme titres, either within or between seasons, higher PBO and profenofos suppression of pyrethroid resistance did broadly correlate with increased levels of cytochrome P450 and esterase activity respectively. The fact that a quantitative change in enzyme activity does not necessarily serve as an indicator of the frequency of inhibitor-suppressible resistance may possibly be attributed to the presence of a number of isozymes expressed at different times in the season or in different populations, with varying degrees of affinity to the pyrethroids and inhibitors. Moreover, PBO is also known to counteract penetration resistance to some extent in *H. armigera*,[10] hence high PBO-suppressible resistance may not be solely associated with metabolic detoxification.

The enzyme assay data indicate that high cytochrome P450 levels were generally coincident with low esterase activity and *vice versa*. Similarly, the synergist bioassays showed that PBO-insensitive resistance was frequently associated with profenofos-sensitive resistance and *vice versa*. It appears therefore that oxidase- and esterase-mediated mechanisms alternated in a reciprocal manner, with perceptible shifts in dominance occurring during mid-October of all three seasons and also in late January in 1995. The regular seasonal shifts in the relative importance of these mechanisms, sometimes within short periods of time, are difficult to account for in terms of changes in local insecticide selection pressure, as there were no obvious systematic changes in farmers' control tactics towards insecticides with different modes of action during each season. While factors contributing to such rapid changes are not properly understood, a plausible explanation may be that influxes of moths from populations subject to different selection pressures contributed significantly to the gene pool at certain times during the season.[8] This would also account for the high pyrethroid resistance in a region of India where this chemical class of insecticides is not that widely used for bollworm control. Such influxes could result from immigration of moths[3,15,18] or emergence of diapause moths[19] from populations exposed to different levels of insecticide selection relative to local populations. As facultative migration[20–22] and diapause[3,19,23,24] have been well documented in *H. armigera* in India, such behaviours may be responsible for, or contribute to, the rapid changes in relative importance of Pbo- versus profenofos-suppressible resistance mechanisms within and between seasons.

Although GST titres in general followed the same seasonal trends as for cytochrome P450, assays with DEM failed to indicate any significant pyrethroid synergism. As DEM is not necessarily a specific inhibitor for GST-mediated resistance,[25] we cannot at this stage make any conclusions as to the likely role, if any, of GSTs in pyrethroid resistance.

Most published reports on metabolic pyrethroid resistance mechanisms have implicated one, or very occasionally two, major mechanisms. For example, a PBO-sensitive resistance has been reported in *Heliothis virescens* (Fab.),[26,27] *H. armigera*[5,9,12,28] and *Pseudoplusia includens* (Walker).[29] This has been attributed to an enhanced mono-oxygenase-mediated resistance in *H. virescens*,[30–32] *H. armigera*,[28,33,34] *Plutella xylostella* (L.)[35] and *Musca domestica* L.[36,37] Some authors confirmed that elevated levels of cytochrome P450 were responsible for this resistance.[30,32,38,39] Profenofos-suppressible, but PBO-insensitive pyrethroid resistance has been reported in *Spodoptera littoralis* (Boisd.), *Trichoplusia ni* (Hübner) and *Chrysopera carnea* (Stephens),[40] *H. virescens*[41] and *P. includens*.[42] A DEF-sensitive pyrethroid resistance has been reported in *S. littoralis*.[43] Elevated esterase levels conferring pyrethroid resistance have been confirmed in *S. littoralis*,[43] *Spodoptera exigua* (Hübner),[44] *H. armigera*,[45] *H. virescens*,[41] *Leptinotarsa decemlineata* (Say)[46] and the cattle tick, *Boophilus microplus* (Canestrini).[47] The majority of authors who have concluded that one particular enzyme-mediated pyrethroid resistance mechanism is predominant have generally done so on the basis of enzyme assays and/or synergist tests conducted on only a few strains either reared in the laboratory for many generations, or collected from the field on only one, or a few occasions during a particular season. As we have clearly demonstrated that resistance mechanisms in *H. armigera* can change markedly over a single cropping season, one-off field collections or assays on laboratory-reared insects, may well fail to identify the true dynamics of metabolic-mediated mechanisms in situations where multiple mechanisms are present. This may account for some of the discrepancies reported by various authors on the relative importance of different mechanisms within the same species, as is the case for example in *H. armigera*,[5,45] *H. virescens*[31,41] and *P. includens*.[29,42]

In most of the published reports where DEF has been used as an indicator of esterase-mediated pyrethroid resistance, either a low or negligible level of synergism has generally been reported. In our studies it was found that esterase resistance was more accurately demonstrated by profenofos rather than DEF, which only indicated a low level of synergism in most weeks (but antagonism in some weeks). Since profenofos is one of the most powerful inhibitors of esterases in a wide variety of insects, compared to other organophosphates,[40,42] it appears to be a more appropriate indicator of the role of esterases in pyrethroid resistance. It is probable that reports based solely on the inefficiency of DEF as a pyrethroid synergist, leading to the conclusion that pyrethroid resistance was not mediated

through esterases, could have underestimated the potential importance of esterases in resistance dynamics.

Although metabolic mechanisms are important factors underlying pyrethroid resistance in *H. armigera* in central India, on average 51, 30 and 28% of recorded cypermethrin resistance in 1993–94, 1994–95 and 1995–96, respectively, was insensitive to either of the two synergists. It is likely that this synergist-insensitive resistance was due to nerve insensitivity, which is present to some degree in *H. armigera* populations in central India.[11] In nearly all weeks, PBO- and esterase-suppressible and synergist-insensitive mechanisms were present together, indicating a very complex pyrethroid resistance situation.

ACKNOWLEDGEMENTS

Financial support was provided by the Natural Resources Institute, UK, through an Adaptive Research Initiative of the UK Government's Overseas Development Administration. We gratefully acknowledge the invaluable technical assistance provided by Mr Sanjay Gaikwad. Comments by Dr R. V. Gunning on an earlier draft are much appreciated.

REFERENCES

1. King, A. B. S., *Heliothis/Helicoverpa* (Lepidoptera: Noctuidae). In *Insect Pests of Cotton*, ed. G. A. Matthews and J. P. Tunstall. CAB International, UK, 1994, pp. 39–106.
2. McCaffery, A. R., King, A. B. S., Walker, A. J. & El-Nayir, H., Resistance to synthetic pyrethroids in the bollworm *Heliothis armigera* from Andhra Pradesh, India. *Pestic. Sci.*, **27** (1989) 65–76.
3. Armes, N. J., Jadhav, D. R., Bond, G. S. & King, A. B. S., Insecticide resistance in *Helicoverpa armigera* in south India. *Pestic. Sci.*, **34** (1992) 355–64.
4. Dhingra, S., Phokela, A. & Mehrotra, K. N., Cypermethrin resistance in the population of *Heliothis armigera* Hübner. *Nat. Acad. Sci. Letters*, II (1988) 123–5.
5. Forrester, N. W., Cahill, M., Bird, L. J. & Layland, J. K., Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bull. Ent. Res. Suppl. Series No. 1* (1993).
6. McCaffery, A. R., Walker, A. J. & Topper, C. J., Insecticide resistance in the bollworm, *Helicoverpa armigera* from Indonesia. *Pestic. Sci.*, **32** (1991) 85–90.
7. Ahmad, M., Gladwell, R. T. & McCaffery, A. R., Decreased nerve sensitivity is a mechanism of resistance in a pyrethroid resistant strain of *Heliothis armigera* from Thailand. *Pestic. Biochem. Physiol.*, **35** (1989) 165–71.
8. Armes, N. J., Banerjee, S. K., DeSouza, K. R., Jadhav, D. R., King, A. B. S., Kranthi, K. R., Regupathy, A., Surulivelu, T. & Venugopal Rao, N., Insecticide resistance in *Helicoverpa armigera* in India: recent developments. *Proc. Brighton Crop Prot. Conf.—Pests and Diseases* **4** (1994) 437–42.
9. Gunning, R. V., Easton, C. S., Balfe, M. E. & Ferris, I. G., Pyrethroid resistance mechanisms in Australian *Heliothis armigera*. *Pestic. Sci.*, **33** (1991) 472–90.
10. Kennaugh, L., Pearce, D., Daly, J. C. & Hobbs, A. A., A piperonyl butoxide synergizable resistance to permethrin in *Helicoverpa armigera* which is not due to increased detoxification by cytochrome P450. *Pestic. Biochem. Physiol.*, **45** (1993) 234–41.
11. West, A. J. & McCaffery, A. R., Evidence of nerve insensitivity to cypermethrin from Indian strains of *Helicoverpa armigera*. *Proc. Brighton Crop Prot. Conf.—Pests and Diseases* **3** (1992) 233–8.
12. Ahmad, M. & McCaffery, A. R., Elucidation of detoxification mechanisms involved in resistance to insecticides in the third-instar larvae of a field-selected strain of *Helicoverpa armigera* with the use of synergists. *Pestic. Biochem. Physiol.*, **41** (1991) 41–52.
13. Phokela, A. & Mehrotra, K. N., Pyrethroid resistance in *Helicoverpa armigera* (Hübner) VI. Effect of PBO on toxicity of cypermethrin. *Pestic. Res. J.*, **5** (1993) 106–8.
14. Armes, N. J., Bond, G. S. & Cooter, R. J., The laboratory culture and development of *Helicoverpa armigera*. *Natural Resources Institute Bulletin 57*. Natural Resources Institute, Chatham, UK, 1992.
15. Armes, N. J., Jadhav, D. R. & Lonergan, P. A., Insecticide resistance in *Helicoverpa armigera* (Hübner): status and prospects for its management in India. *In Challenging the Future, Proc. World Cotton Conf.—1*, Brisbane, Australia, 13–17 Feb. 1994, ed. G. A. Constable and N. W. Forrester. CSIRO Australia, 1995, pp. 522–33.
16. Lowry, D. H., Rosebrough, A. L. & Randall, R. J., Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.*, **193** (1951) 265–75.
17. Omura, T. & Sato, S., The carbon monoxide binding pigment of liver microsomes. II. Solubilisation, purification and properties. *J. Biol. Chem.*, **239** (1964) 2379–85.
18. Madden, A. D., Holt, J. & Armes, N. J., The role of uncultivated hosts in the spread of pyrethroid resistance in *Helicoverpa armigera* populations in Andhra Pradesh, India: a simulation approach. *Ecol. Modelling*, **82** (1995) 61–74.
19. DeSouza, K. R., Holt, J. & Colvin, J., Diapause, migration and pyrethroid-resistance dynamics in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Ecol. Entomol.*, **20** (1995) 333–42.
20. Pedgley, D. E., Tucker, M. R. & Pawar, C. S., Windborne migration of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in India. *Insect Sci. Applic.*, **8** (1987) 599–604.
21. Riley, J. R., Armes, N. J., Reynolds, D. R. & Smith, A. D., Nocturnal observations on the emergence and flight behaviour of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in the post-rainy season in central India. *Bull. Ent. Res.*, **82** (1992) 243–56.
22. Armes, N. J. & Cooter, R. J., Effects of age and mated status on the flight potential of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Physiol. Entomol.*, **16** (1991) 131–44.
23. Bhatnagar, V. S. & Davies, J. C., Factors affecting populations of gram podborer, *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in the period 1974–77 at Patancheru, Andhra Pradesh. *Bull. Entomol.*, **19** (1978) 52–64.
24. Tripathi, S. R. & Singh, R., Seasonal bionomics of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in the Terai belt of north eastern Uttar Pradesh. *Insect. Sci. Applic.*, **14** (1993) 439–44.
25. Price, N. R., Insect resistance to insecticides: mechanisms and diagnosis. *Comp. Biochem. Physiol.*, **100** (1991) 319–26.
26. McCaffery, A. R., Little, E. J., El-Nayir, H. & Holloway, J., Toxicity and synergism of pyrethroids in various life history stages of the tobacco budworm (*Heliothis virescens*). In *Progress and Prospects in Insect Control*. British Crop Prot. Coun. Monograph No. 43, 1989, p. 263.

27. Bagwell, R. D. & Plapp, F. W., Synergism of insecticides against susceptible and pyrethroid-resistant tobacco budworms (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, **85** (1992) 658–63.
28. Xiao Bin, The preliminary biochemical analysis of resistance of the cotton bollworm in China. *Resistant Pest Management Newsletter*, **6** (1994) 4–5.
29. Thomas, J. D. & Boethel, D. J., Synergism of insecticides in tests with resistant soybean looper larvae (Lepidoptera: Noctuidae) in the laboratory and field. *J. Econ. Entomol.*, **87** (1994) 1416–22.
30. Clarke, S. E., Walker, C. H. & McCaffery, A. R., A comparison of the *in vitro* metabolism of *cis*-cypermethrin in a resistant and susceptible strain of *Heliothis virescens*. *Proc., Brighton Crop Prot. Conf.—Pests and Diseases* (1990) 1201–6.
31. Little, E. J., McCaffery, A. R., Walker, C. H. J. & Parker, T., Evidence for an enhanced metabolism of cypermethrin by a mono-oxygenase in a pyrethroid-resistant strain of the tobacco budworm (*Heliothis virescens*). *Pestic. Biochem. Physiol.*, **34** (1989) 58–68.
32. Lee, K. S., Walker, C. H., McCaffery, A. R., Ahmad, M. & Little, E., Metabolism of trans-permethrin by *Heliothis armigera* and *Heliothis virescens*. *Pestic. Biochem. Physiol.*, **34** (1989) 49–57.
33. Daly, J. C., Insecticide resistance in *Heliothis armigera* in Australia. *Pestic. Sci.*, **23** (1988) 165–76.
34. Phokela, A., Dhingra, S. & Mehrotra, K. N., Pyrethroid resistance in *Heliothis armigera* Hübner. Permeability and metabolism of cypermethrin. *Proc. Nat. Acad. Sci. India*, **55** (1989) 235–8.
35. Hung, C. F. & Sun, C. N., Microsomal mono-oxygenases in diamondback moth larvae resistant to fenvalerate and piperonyl butoxide. *Pestic. Biochem. Physiol.*, **33** (1989) 168–75.
36. Goldena, C. F. & Forgash, A. J., The distribution and metabolism of fenvalerate in pyrethroid resistant and susceptible houseflies. *Pestic. Biochem. Physiol.*, **33** (1989) 37–48.
37. Devries, D. H. & Georghiou, G. P., Absence of enhanced detoxification of permethrin in pyrethroid resistant houseflies. *Pestic. Biochem. Phyisol.*, **15** (1981) 242–52.
38. Wheelock, G. D. & Scott, J. G., The role of cytochrome P450 in deltamethrin metabolism by pyrethroid resistant and susceptible strains of houseflies. *Pestic. Biochem. Physiol.*, **43** (1992) 67–77.
39. Chen, Y. Q., Yan, F. & Sun, Y. Q., Influence of aldrin epoxidationase and cytochrome P450 on the development of insecticide resistance in the diamondback moth, *Plutella xylostella* L. *Acta. Entomol. Sinica*, **37** (1994) 280–5.
40. Ishaaya, I. & Casida, J. E., Pyrethroid detoxification and synergism in insects. In *IUPAC Pesticide Chemistry: Human Welfare and the Environment*, ed. J. Miyamoto and P. C. Kearney, Vol. 3. Pergamon Press, UK, 1983, pp. 307–10.
41. Dowd, P. F., Gagne, C. G. & Sparks, T. C., Enhanced pyrethroid hydrolysis in pyrethroid resistant larvae of the tobacco budworm, *Heliothis virescens* (F.). *Pestic. Biochem. Physiol.*, **28** (1987) 9–16.
42. Dowd, P. F. & Sparks, T. C., Inhibition of *trans*-permethrin hydrolysis in *Pseudoplusia includens* (Walker) and use of inhibitors as pyrethroid synergists. *Pestic. Biochem. Physiol.*, **27** (1987) 237–45.
43. Riskallah, M. R., Esterases and resistance to synthetic pyrethroids in the Egyptian cotton leafworm. *Pestic. Biochem. Physiol.*, **19** (1983) 184–9.
44. Delorme, R., Fournier, D., Chaufaux, J., Cuany, A., Bride, J. M., Danielle, A. & Berge, J. B., Esterase metabolism and reduced penetration are causes of resistance to deltamethrin in *Spodoptera exigua* Hub. (Noctuidae: Lepidoptera) *Pestic. Biochem. Physiol.*, **32** (1988) 240–6.
45. Gunning, R. V., Esterases and pyrethroid resistance in Australian *Helicoverpa armigera*. *Resistant Pest Management Newsletter*, **6** (1994) 8–9.
46. Solomennikova, I. B., Nedel Kina, N. S., Lukashina, I, Raushenbakh, Yu. & Salganik, R. I., The role of esterases in the development of resistance to permethrin in the colorado beetle: ontogenic and population aspects. *Agrokhimiya*, **9** (1990) 137–42.
47. Dejersey, J., Nolan, J., Davey, P. A. & Ridles, P. W., Separation and characterization of the pyrethroid hydrolyzing esterases of the cattle tick *Boophilus microplus*. *Pestic. Biochem. Physiol.*, **23** (1985) 349–57.